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Short communication

Micropreparation of hemopoietic stem cells from the mouse bone marrow suspension by gravitational field-flow fractionation

Eva Urbánková[a,*], Antonín Vacek[b], Josef Chmelík[a]

[a]*Institute of Analytical Chemistry, Academy of Sciences of the Czech Republic, Veveří 97, 61142 Brno, Czech Republic*
[b]*Institute of Biophysics, Academy of Sciences of the Czech Republic, Královopolská 135, 61265 Brno, Czech Republic*

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Abstract

Gravitational field-flow fractionation is a relatively simple experimental technique. This method was used for the characterization of stem cells from mouse bone marrow. Because these cells are bigger than the other cells in bone marrow, it is possible to separate them from the mixture. The fractions collected after passing through the separation channel were characterized using a Coulter Counter and used for transplantation into irradiated mice.

Keywords: Hemopoietic stem cells; Field-flow fractionation; Gravitational field-flow fractionation

1. Introduction

There are several reasons for the isolation of subpopulations of living cells (preparation of pure cells for transplantation, selection of fused cells, product analysis etc.), and for these purposes various methods can be used (centrifugation, electrophoresis, field-flow fractionation, affinity methods, biphasic extraction, etc.) [1].

Gravitational field-flow fractionation (GFFF) is a suitable technique for the separation of bone marrow cells because experimental conditions can be chosen to be gentle enough to maintain the integrity and function of the biological structures. This technique makes it possible to prepare relatively concentrated samples of fractionated cells under physiological conditions, and living cells are separated according to size, density and shape. The separated cells can be used for further biological investigations. The other advantage of this method is its simplicity. GFFF utilizes Earth's gravity as an external force field which causes settlement of particles toward the channel bottom [2,3]. However, hydrodynamic lift-forces also act on particles in the carrier liquid flow [4]. In contrast to the gravitation, they tend to drive particles away from the channel accumulation wall and to focus them into narrow zones. These zones are located in the flow velocity profile according to particle size and density due to the simultaneous action of the gravitational force field and hydrodynamic lift-forces. GFFF has been used for the separation of glass beads [2], chromatographic silica-gel supports [5–8], polystyrene latexes [9] and for the characterization of blood cells [10–17].

For biological studies it is often necessary to prepare relatively pure subpopulations of cells. This paper describes the micropreparation of stem cells from mouse bone marrow by GFFF. The aim of the

*Corresponding author.

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work was to obtain fractions of stem cells in amounts which can be used for further biological applications. The fractions were analyzed using a Coulter Counter and were applied to irradiated mice.

2. Experimental

2.1. Device and procedure

The separation channel was cut in a spacer which was placed between two mirror-quality float glasses and clamped between two plexiglass blocks. The dimensions of the channel were: width, 20 mm; length, 360 mm; height, 0.08 mm. The samples were injected into the separation channel using a microsyringe by an injection system with a septum placed directly in front of the inlet capillary. The carrier liquid was introduced into the channel by a HPP 4001 pump (Laboratory Instruments, Prague, Czech Republic). The flow-rate was maintained at 200 μl/min during the injection for 6 s. Then the flow was stopped for the period of time necessary for relaxation (1 min). After this time, the flow-rate of 100 μl/min was reapplied. The concentration of the sample was 2×10^7 cells/ml. The injected volume was 40 μl. The carrier liquid was Iscove's modification of Dubelcco's medium (IMDM) (Catalog No. I 7633, with L-glutamine, 25 m*M* HEPES buffer and without sodium bicarbonate; Sigma Chemical Co., St. Louis, MO, USA). Originally this cell culture medium was used for precursor cells of erythrocytes and macrophages and the applicability of IMDM has been extended to include hemopoietic tissue from bone marrow, B-cells stimulated with liposaccharide, T-lymphocytes and a variety of hybrid cells. A VD 104 detector (Labeco, Poprad, Slovak Republic) at 565 nm was used for the detection of particles.

2.2. Experimental animals

Conventional (CBAx C57B/10) F1 mice, three months old and with an average body weight of 25 g, were used. A standardized pellet diet and HCl-treated tap water (pH 2–3) were given to the mice.

2.3. Hematological methods

Bone marrow of mice was prepared in IMDM supplemented with 5% fetal bovine serum at the temperature of melting ice. The number of nuclear cells in suspension was measured using a Coulter Counter. For these measurements only a small amount from each fraction was used and it was diluted 100 times.

After suitable dilution, the cell suspension (10^5 cells) was injected into the lateral caudal veins of 10 syngeneic recipients of 1 h gamma irradiation at a dose of 9.0 Gy. Ten days after injection, the mice were euthanized by cervical dislocation, their spleens were removed, fixed in Bouin's solution and the nodules greater than 0.4 mm were counted [18]. The same procedure was used for injection of prepared fractions.

3. Results and discussion

In spite of being an experimentally simple technique, GFFF is a powerful tool having the capacity for high-resolution separations of various kinds of particles. This technique is often used for the study of biological particles and cells [10–17]. Micropreparation of stem cells from bone marrow is important for transplantation.

The hemopoietic stem cells are multipotent and are responsible for the generation of all recognizable hemopoietic cells. After injection of small numbers of stem cells into mice, where the hemopoietic populations were destroyed by gamma irradiation, discrete macroscopically visible surface colonies appear in the spleen in the 10 days after injection. It was demonstrated that each colony was a clone derived from a single initiating cell [18]. This technique was used for the characterization of prepared fractions of stem cells.

Fractionation of mouse bone marrow cells at the flow-rate of 100 μl/min is shown in Fig. 1. An eluted sample was separated into six fractions and these were characterized by the number of cells and the mean cell volume (MVC) using a Coulter Counter (Table 1 and Fig. 1). It is evident that the stem cells are mainly in the first fraction because their size is bigger than the size of the other cells.

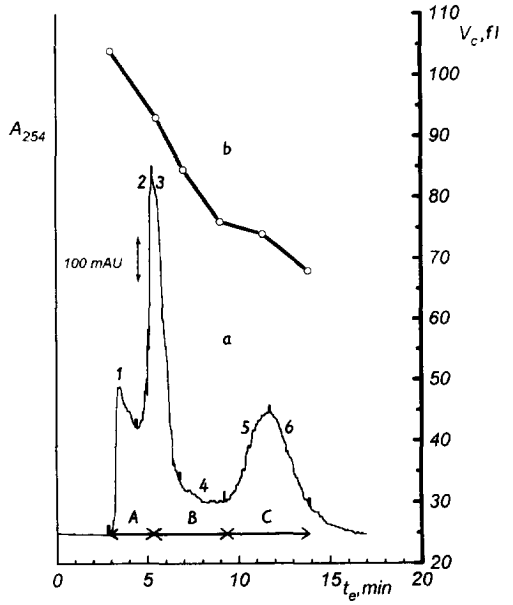

Fig. 1. Separation of stem cells from mouse bone marrow. (a) Fractogram of mouse bone marrow with marked fractions 1–6. (b) Mean cell volumes of fractions 1–6 measured using a Coulter Counter. A, B and C denote fractions used for transplantation. Carrier liquid, IMDM solution; flow-rate, 100 μl/min; sample concentration, 2×10^7 cells/ml; sample volume, 40 μl; relaxation time, 1 min; detection wavelength, 254 nm.

The concentration of the stem cells decreases in further fractions. The fractions 2 and 3 contain beside the stem cells some smaller particles and macromolecules. The fractions 4–6 consist mainly of red blood cells. The retention of these fractions corresponds to the retention of red blood cells [11,12,17].

These facts were proved by the quantity of colonies in the spleens of mice. For the experiment, three fractions A, B and C (Fig. 1) were taken from the sample after elution from the channel and 10^5 cells from every fraction were given to irradiated mice. The results of the experiments (Table 2) document the highest number of spleen colonies in the group of mice to which the first collected fraction A was given. In this group the number of colonies and the mass of the spleen are statistically significantly higher ($p<0.01$) than that of the control group injected with an initial suspension of bone marrow. In the group of mice to which the fraction B was given, the number of colonies is close to that of the control group. In the group with the fraction C injected, no colonies were observed.

The results of the transplantation procedure indicate that the concentration of hemopoietic stem cells in the fraction A is relatively high and the quality of purified cells is suitable for further biological applications. Because the separation can be performed under physiological conditions in a short time, we can conclude that GFFF is a suitable method for the micropreparation of living cells.

Table 1
Characterization of the individual separated fractions of mouse bone marrow

Fraction	Number of particles	Mean cell volume (fl)
1	1594	104
2	1672	93
3	1167	85
4	807	76
5	675	74
6	732	68

The fractions and the experimental conditions are described in Fig. 1.

Table 2
Number of spleen colonies of mice treated with three different fractions of microprepared cells from bone marrow

Fraction	Mean cell volume (fl)	Mass of spleen (mg)	Number of colonies
A	98	82.00±4.76	18.75±1.26
B	84	61.66±1.23	13.30±2.40
C	72	13.25±0.84	0.00
control	76	54.30±4.86	11.66±1.26

Control is a group of mice treated with the original bone marrow suspension.

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